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CMV infections cause significant morbidity & mortality, particularly among HLA mismatched HSCT recipients. Adoptive immunotherapy using donor derived CMV-Specific cytotoxic T-cells (D-CMV-CTLs) can treat CMV infection in such patients. However, D-CMV-CTLs cannot be generated for HSCT recipients from CMV seronegative donors, and generation of such T-cells (TC) from cord blood has been difficult. Furthermore, D-CMV-CTLs in recipients of HLA disparate HSCT maybe ineffective if the HLA allele restricting their cytotoxic activity is not shared between the donor and host. In these conditions, persistent CMV reactivation can be treated using pre-generated CMV-CTLs of defined epitope specificity and HLA restriction from third party donors.

Many HLA antigens (ags) have multiple allelic variants. We asked if TC specific for epitopes of CMVpp65 presented by one allelic variant might also recognize the same epitope presented by other variants of the same HLA ag. We characterized the epitope specificities and HLA restrictions of 105 CMV-CTLs generated and banked for a phase I clinical trial, plus 41 additional donors. CMV-CTLs in 37 HLA A0201^{+} donors were consistently responsive to the A0201 presented NLV epitope. These NLV responsive TC from HLA A0201^{+}donors were also cytotoxic against NLV loaded targets expressing HLA A0201, 0205 and 0206, but not HLA 0202, 0211 and 0220. In 13 donors expressing HLA B40, B44 and B42 ag, CMV-CTLs were responsive to the HERNGFTVL epitope when presented by APCs expressing any one of the following alleles: HLA B 4001, 4002, 4006, 4401, 4403 or 4202. Importantly, CTLs from any one of these donors were also cytotoxic against HERNGFTVL loaded targets bearing one each of these alleles. TC from 9 donors inheriting HLA B35, all elicited responses to the epitope EVQAIRETVE which could be presented by either HLA B3501, 3502, 3503, 3508 or 3511; each CTL line was also cytotoxic against EVQAIRETVE loaded targets bearing one of these HLA B35 alleles. Similar cross reactivity was demonstrated for the QYDPVAALF epitope which can be recognized when presented by HLA A2402, 2404 and A2407. In contrast, allelic variants for a given HLA class-II allele do not share epitope specific responses in all donors; e.g. T-cells responding to the FTSQYRIQGKL are cytotoxic against peptide loaded DRB1 1101 targets, but do not lyse FTSQYRIQGKL loaded DRB1 1104 targets; DRB1 1104 presents a different epitope VAALFFFDIDL.

Our findings indicate that certain immunodominant viral epitopes may be presented by multiple allelic variants of class-I HLA ags. These data suggest that HLA class-I restricted epitope specific CMV-CTLs from HLA disparate donors can be effective in recipients sharing certain allelic variants within the same class-I family. Thus, generation of a bank of CTLs responding to common immunodominant epitopes could also potentially be used for treatment of a broader patient population at risk for CMV.

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Safe and Effective Treatment of Graft Versus Host Disease with Platelet Lysate-Expanded Mesenchymal Stromal Cells: A Prospective, Multicentric, Phase 1 Study

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Background: Conventional steroid treatment for graft versus host disease (GvHD) is effective for a limited number of patients (pts). The use of mesenchymal stromal cells (MSC) as second line of treatment has been reported with promising results. We assessed the safety and efficacy of MSC, in a prospective, multicenter, phase I study (EudraCT 2008-007869-23) for the treatment of steroid resistant grade II-IV GvHD.

Methods: MSC were obtained from bone marrow harvests of third party donors and expanded with platelet lysate. Primary endpoint of the study was safety. Secondary endpoints were response to MSC, overall survival (OS) and transplant-related mortality (TRM). Plasma levels of IL2Ralpha by ELISA were analyzed for enrolled pts.

Results: Between August 2009, and June 2012, 16 children and 31 adults were treated. The median dose of infused MSC was 1.5×10^{6} cells per kg. Enrolled pts presented with acute GvHD in 37 cases, chronic overlap syndrome in 7, and chronic classic GvHD in 3 cases. 15 pts had grade II GvHD, 23 grade III and 9 grade IV, according to NIH criteria. In 17 cases GvHD involved a single organ. Pts were treated with a median of 3 MSC infusions. No significant side effects were registered. 30 patients (63.8%) showed a clinical response. 13 of them (27.6%) had a complete response and 17 (36.1%) a partial response to treatment. 22 over 30 responding pts did not require further lines of immunosuppression after MSC infusion. Response was more likely in pts exhibiting grade II GvHD versus those with severer grading (87.5% vs. 51.6%, $P = .02$) and in pts receiving MSC within 30 days from GvHD onset (75.9% vs. 43.7%, $P = .05$). Median follow up for this cohort is 200 days (range 30-1066). Responders show a significant lower TRM (10.0% vs. 88.2%, $P < .05$) and a better OS probability than non responders (23.3% vs. 88.2%, $P < .05$). When comparing responders vs non-responders, IL2Ralpha showed a statistically significant difference in terms of fold decrease (p=0.027), corroborating clinical results. Similarly, a significant trend of fold decrease (p=0.058) was observed when comparing responder patients receiving MSC within or after 30 days from GvHD onset, in line with clinical results.

Conclusions: This study confirms that MSC may represent a safe and effective treatment for patients with steroid-refractory GVHD. Plasmatic markers may help in monitoring of clinical response. We suggest to consider the use of MSC as early as possible, after steroid failure.

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CD25+ Regulatory T CELL-Depleted Donor Lymphocyte Infusion for Relapse After Allogeneic Transplantation – A PHASE 1 Study

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